OBSERVATIONS ON THE GENITAL SEGMENTS OF SPIRORBIS (POLYCHAETA) [1]

HERBERT E. POTSWALD [2]

Department of Zoology, University of Washington, Seattle, Washington 98105

The serpulid genus *Spirorbis* has attracted the attention of numerous investigators for more than a century. Pagenstecher (1863) noted that *Sp. pagenstecheri* exhibited brood protection and was hermaphroditic. In subsequent accounts (Agassiz, 1866; Fewkes, 1885; Schively, 1897; Bush, 1904; Elsler, 1907; zur Loye, 1908; Borg, 1917; and others) Pagenstecher's observations have been confirmed for all species examined. To date, the most comprehensive study on the morphology and reproductive biology of *Spirorbis* is that of Bergan (1953a). Bergan's study, although contributing valuable information, leaves many questions unanswered and fails to give an adequate description of the genital segments. The observations presented here were made in conjunction with an embryological study of *Spirorbis* and serve to add new information concerning the morphology and nature of the genital segments in this complex and enigmatic genus.

Materials and Methods

Collection and maintenance of adult animals

Spirorbis (*Laeospira*) *mörchi* Levinsen, *Sp.* (*Paradexiospira*) *vitreus* Fabricius, and *Sp.* (*Dexiospira*) *spirillum* Linné were collected intertidally on San Juan Island, Washington, periodically throughout the year, from 1960 to 1963. *Spirorbis spirillum* was also frequently collected on hydroid colonies (*Abietinaria* sp.) dredged 10–23 fathoms off San Juan Island. *Spirorbis* (*Protolaeospira*) *ambilateralis* Pixell was collected by dredging off San Juan Island and was found most frequently on *Modiolus modiolus*, *Balanus nubilis*, and *Chlamys* sp., and often in association with *Sp. vitreus*. The system of classification followed is that first used by Caullery and Mesnil (1897) and later adopted by Fauvel (1927). The identities of the species reported on here were determined from descriptions given by Bush (1904), Pixell (1912), Fauvel (1927), Berkeley and Berkeley (1952), Bergan (1953b), and Pettibone (1954). In an examination of the Washington species, the procedure was first to prepare a species description, as complete as possible, before going to the literature to make comparisons. Of the several hundreds of specimens examined, *Sp. mörchi* and *Sp. ambilateralis* have always been found to be sinistral and *Sp. spirillum* dextral. On the west side of San Juan Island, Washington, however, there are extensive populations of dextral and sinistral *Sp. vitreus*. The sinistral form occurs together with the dextral form in about

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[2] Present address: Department of Zoology, University of Massachusetts, Amherst, Massachusetts 01002.

equal numbers. They are exactly alike in all diagnostic characters except the direction of coil. Observations on isolated brooding adults indicate that this is a case of true genetic polymorphism (Potswald, 1965).

The animals were kept in the circulating sea water system at the Friday Harbor Laboratories, or were brought to the Seattle campus where they were kept in the Zoology Department's 10° C. cold room. In Seattle, no attempt was made to provide food; however, the animals remained in good condition for several months at a time, and brooding individuals were always available.

Adult animals were observed both within their tubes and removed from their tubes. In the latter case, animals were removed from their substratum by means of a razor blade and then removed from their tubes by chipping away the calcareous secretion with dissecting needles. This is a relatively easy task except for *Sp. vitreus,* which has an extremely hard tube.

Microtechnique

Adult worms removed from their tubes were fixed in a variety of fixatives including: Bouin's fluid, Helly's with and without post-chroming, buffered formalin, Flemming's with and without acetic acid, and Carnoy without chloroform. Fixed material was dehydrated through ethyl alcohol and tertiary butyl alcohol, and embedded in a mixture of 300 gm. of Fisher's Tissuemat (M.P. 60–62° C.) and 45 gm of dry piccolyte. This is the mixture recommended by Cloney (1961) but without beeswax. Blocks, chilled in ice-water, were sectioned 5–6 microns at room temperature.

A number of stains were used including Heidenhain's iron haematoxylin, Heidenhain's Azan, and Harris' or Ehrlich's haematoxylin with eosin counterstain. Feulgen and PAS methods were followed according to McManus and Mowry (1960).

In addition to routine paraffin technique, material was fixed in cold buffered osmium tetroxide (Bennett and Luft, 1959) and embedded in Epon 812 according to the method of Luft (1961). Thin sections, ½–1 micron, were cut on a Porter-Blum ultra-microtome, using glass knives. After affixing to glass slides, the sections were stained with Richardson's stain (Richardson *et al.,* 1960) for study with the light microscope.

Description of Genital Segments

Bergan (1953a), from his study of *Sp. borealis,* concluded that *Spirorbis* is a simultaneous hermaphrodite and not protandrous as suggested by Hempelmann (1931). Although providing histological evidence for his conclusion, Bergan fails to give an adequate description of the genital segments. Aside from Franzén's study (1956, 1958) on late stages of spermiogenesis and Dasgupta and Austin's (1960) examination of chromosome numbers in spermatocytes, little information concerning the gametes and their development in *Spirorbis* is available.

The observations presented here are based, for the most part, on a study of *Sp. mörchi.* Only where differences in detail have been found, will the other three species be mentioned.

Female Segments

Normal arrangement and anomalies

Germ cell proliferation is restricted to the abdominal or secondary segments; the thoracic or primary segments never serve in this capacity. In a mature adult every abdominal segment contains germ cells in various stages of formation. Each genital segment contains a large coelomic space and is separated from its neighbors by complete septa. In *Sp. mörchi* the first two abdominal segments are female and the remaining abdominal segments male (Fig. 2). *Sp. ambilateralis* presents the same arrangement, but the left halves of segments one and two are generally larger than the right halves of the same segments. Of the dextral species, *Sp. vitreus* is like *Sp. mörchi*, while *Sp. spirillum* presents quite a different picture. In local populations of *Sp. spirillum*, either the first three segments or first $3\frac{1}{2}$ segments are female; the left half of the fourth segment in the latter condition is male. The anomalous condition is the most prevalent. According to Bergan (1953a), specimens of *Sp. spirillum* collected in Oslofjord exhibit lateral asymmetry in sex differentiation similar to that described above; therefore, it would seem that such anomalous sex differentiation is common in *Sp. spirillum*.

Although one might expect to find anomalies in a simultaneous hermaphrodite such as *Spirorbis*, sex differentiation within the genus, with the notable exception of *Sp. spirillum*, seems to be under rigid control and a specific arrangement of female and male segments prevails for a given species. After examination of several hundred specimens of adult *Sp. mörchi*, only a few anomalies have been observed. Lateral asymmetry was found in one animal in which the left half of the second segment was male and the right half female. One case was observed in which the first three abdominal segments were female instead of the first two as is the normal condition. Finally, in a few instances, individuals were found to have sperm and oocytes developing together in the same segment. The latter condition generally occurs in the posterior male segments where the infrequent oocytes have never been observed to enter into vitellogenesis. Only in two individuals have oocytes and sperm been observed to develop together in the second abdominal segment, between a purely female and a male segment (Fig. 3). Oocytes developing in the posterior part of the achaetous zone, as described by Bergan (1953a) for *Sp. borealis*, have not been found.

Development of the primary oocyte

The gonad is a discrete and constant organ composed of clumps of cells arranged in two retroperitoneal rows, mesial to the ventral nerve cords, and running the length of the abdominal segments (Figs. 4, 5, 6, 7). In sexually mature adults, the gonad is always larger in the female segments than in the male segments where it is greatly compressed against the ventral ectoderm.

An examination of the gonad in the female segments reveals the presence of a number of primordial germ cells in interphase. While in interphase, these are the most distinctive cells in the adult body. The cells have almost spherical nuclei 5 to 6 microns in diameter, and the cytoplasm is reduced to a mere envelope. Scattered around the periphery of the nucleus, in a predictable pattern, are spherical

clumps of Feulgen-positive chromatin; the center of the nucleus resists staining and appears transparent (Fig. 1a). Counts, made from thick sections, indicate that there are ten chromatin clumps per nucleus, this number apparently being constant. A nucleolus is absent. The cytoplasm is so reduced that little detail can be resolved in it with light microscopy.

Since the gonad is the site of constant proliferation, the primordial germ cells are generally observed in various stages of mitosis. As nuclei enter prophase, the thread-like projections appear to radiate out from the individual chromatin spheres and the "unraveling" continues until the nucleus is completely occupied by typical prophase chromosomes (Fig. 1a). Metaphase, anaphase, and telophase follow in an orderly fashion. The telophase nuclei of the resulting daughter cells are small and vesicular, with the diameter of the nuclei measuring about 2.5 microns and that of the entire cell about 3.2 microns (Fig. 1b). It is assumed that the mitotic divisions give rise either to more primordial germ cells which resume the characteristic interphase condition or to a morphologically different cell type, the early primary oocyte. The term "primordial germ cell" has been used for the proliferating cell since a distinct gonial stage has not been recognized.

FIGURE 1. *Sp. mörchi:* (1a) section through primordial germ cells in interphase (I), and prophase (Pr); (1b) section through four primordial germ cells having just completed telophase (T), and one primordial germ cell undergoing telophase reconstruction (TR). Drawing made from a photomicrograph. Paraffin; Feulgen. (1880 ×)

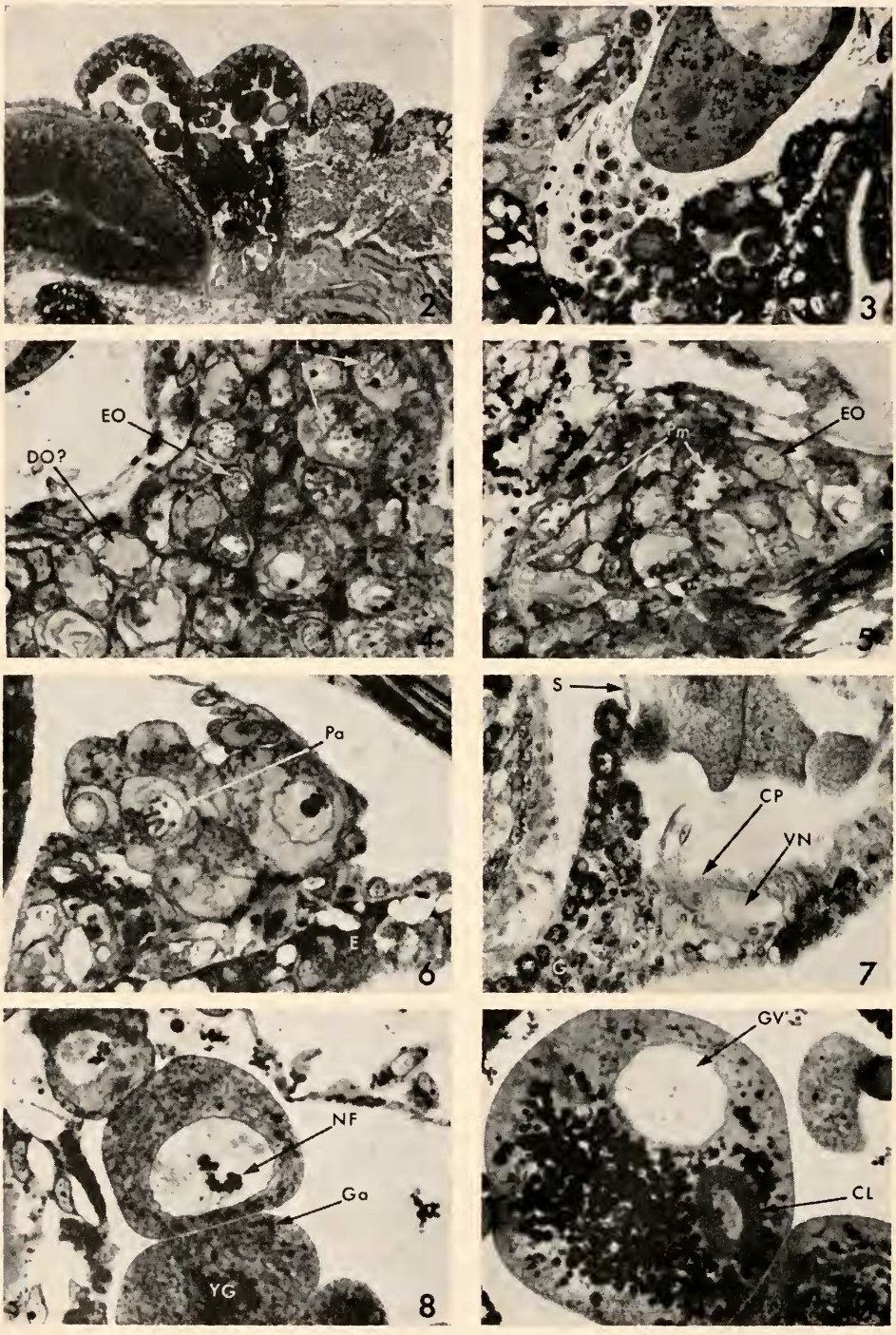
Early primary oocytes have a nuclear diameter of about 6 microns and a cytoplasmic component somewhat larger than that found in the primordial germ cell. The nucleus contains a single nucleolus and a diffuse chromatin network (Figs. 4, 5). Premeiotic stages of prophase are passed within the gonad proper. The delicate chromatin net of the interphase nucleus becomes coarser and more irregular and, with further condensation, chromatin clumps appear not unlike those found in the primordial germ cell. The nucleus has now become elliptical in shape and measures about 7.5 microns in length (Fig. 5). Following condensation, the chromatin bodies become frayed and give rise to delicate chromosomal threads. As concerns succeeding stages of premeiotic prophase, only leptotene and early pachytene, still in "bouquet" arrangement, have been observed (Figs. 4, 6). Scattered throughout the gonad are a number of cells about the same size as oocytes in meiotic prophase; however, unlike the normal oocytes, these cells have structureless nuclei often irregular in outline (Fig. 4). The role of the latter cells in normal development is not clear, but it is possible that they are degenerating germ cells.

With the conclusion of the events of premeiotic prophase, the nucleus again enters the diffuse or "confused" state and regains its spherical shape. A distinct

nucleolus reappears along with several irregularly shaped pieces of chromatin; the basophilic cytoplasmic portion has increased and the diameter of the cell is now about 11 microns. Oocytes of the latter size-class are found beneath the bulging peritoneal covering of the gonad and at the base of the septa. As the oocytes increase in size, they invade the septum and a progressive series can be found extending dorsally from the gonad. The oocytes are located between the two thin epithelial layers making up the septum (Fig. 7). While within the septum, the cytoplasm of the oocytes is strongly basophilic, and little or no yolk deposition occurs. In nuclei attaining a diameter of 13 microns, the single nucleolus starts to fragment into a number of small Feulgen-negative bodies. Cells having this nuclear diameter are about 22 microns in diameter and are starting to erupt into the coelom. Their cytoplasm is filled with coarse membrane, a few scattered proteid yolk granules, and numerous, small, spherical mitochondria (Fig. 8). In thin sections of osmium-fixed material, small rod-like bodies about one micron long, staining intensely with Richardson's stain, are found in the cytoplasm around the nucleus. The bodies are often crescent-shaped and may represent Golgi material (Fig. 8).

The bulk of vitellogenesis takes place in oocytes which have erupted through the septum, and are floating free within the spacious coelom. There are no nurse cells, as such, nor follicle cells associated with the oocytes. As cytoplasmic mass increases, nuclei, which also steadily increase in size, become more eccentric in position. In cells measuring 39 × 33 microns and having a nuclear diameter of about 22 microns, yolk granules begin to arise in a rather localized area within the cytoplasm. At the start of vitellogenesis, and even at its close, yolk granules give only a slight PAS reaction which is not qualitatively affected by incubation in diastase. Associated with the yolk granules are several lamellar stacks of membrane. The membrane stacks are very sensitive to the fixative employed and have been observed only in material fixed in either buffered osmium or Helly's fluid followed by post-chroming. As oocytes approach a diameter of about 50 microns, the lamellar stacks become concentric, thereby enclosing an internum. Clusters of mitochondria are associated with the concentric bodies, and both proteid and lipid yolk granules occur within the internum (Fig. 9). The description given here corresponds in many respects to those given by several authors for structures termed "yolk nuclei," which have been observed in various groups of animals (see Raven, 1961, for survey). As vitellogenesis proceeds and the cytoplasm becomes filled with yolk granules, the concentric structures disappear; their fate has not been followed. There is little visible differentiation of the cortex below the PAS-positive vitelline membrane, cortical granules apparently being absent.

Maximum growth has been attained, and the primary oocyte is ready to be spawned, when it measures about 165 microns × 132 microns. From the start of vitellogenesis, yolk granules have increased in diameter from a fraction of a micron, in the case of proteid yolk, to about 8 microns. The germinal vesicle has become very wrinkled in appearance and has an average measurement of 26 × 13 microns. Nucleolar fragments, so abundant during vitellogenesis, have become reduced both in number and in size. The germinal vesicle apparently does not break down until after spawning has occurred. Animals ready to spawn contain



FIGURES 2–9.

two size-classes of oocytes in their coeloms: full grown primary oocytes and early primary oocytes still within or attached to the septa. Animals have been observed to spawn within 12 hours after releasing larvae, and since it takes about 30 days from fertilization to larval release at 12° C., it is assumed that vitellogenesis occupies about the same period of time.

From the present study, it is difficult to come to any definite conclusions as to the origin of yolk. Nucleolar fragmentation, concentric lamellae, and possible Golgi material have been mentioned, and it is conceivable that they all participate in yolk formation. At the onset of vitellogenesis, the peritoneal cells lining the coelomic cavity start to accumulate large droplets, both lipoidal and proteid in nature, within their cytoplasm. The droplets accumulate to such a degree as to cause the ordinarily flattened peritoneal cells to bulge into the coelomic cavity (Fig. 10). Apical portions of cells actually bud off and become free in the coelom. Towards the end of vitellogenesis, the peritoneal cells again become flattened and relatively devoid of inclusions. There is no evidence that the oocytes are phagocytic; however, correlation between the onset and decline of the storage phenomenon in peritoneal cells with that of vitellogenesis would seem to suggest that transfer of material in some form takes place. Conceivably, such transfer could be in the form of high molecular weight compounds. Finally, it should be mentioned that it is not uncommon for one or two oocytes per segment to disintegrate midway through vitellogenesis. It is not known what role, if any, this phenomenon plays during normal development of the primary oocyte.

The above outline of events leading to the development of the primary oocyte in *Sp. mörchi* also holds true, in the main, for the other three species studied. In *Sp. vitreus,* the behavior of the nucleolus is quite different from that in *Sp. mörchi.*

FIGURE 2. *Sp. mörchi:* parasagittal section through the abdomen of a typical adult, showing that the first two abdominal segments are female and the remaining abdominal segments are male. Epon; Richardson's stain. (110 ×)

FIGURE 3. *Sp. mörchi:* parasagittal section through the second abdominal segment of an adult, showing the simultaneous development of oocytes and spermatids within the same segment. Epon; Richardson's stain. (700 ×)

FIGURE 4. *Sp. mörchi:* frontal section through the gonad in a female segment showing presumably degenerating oocytes (DO?), early oocytes (EO), and leptotene (L). Epon; Richardson's stain. (800 ×)

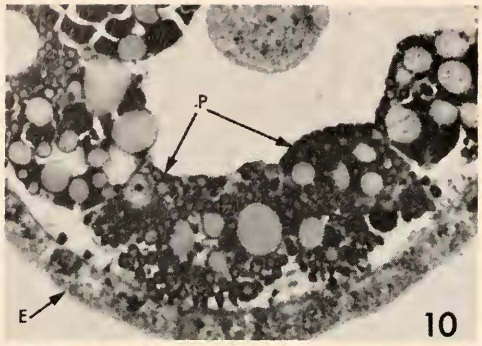
FIGURE 5. *Sp. mörchi:* frontal section through the gonad of a female segment showing early oocytes (EO) and a premeiotic oocyte (Pm) containing condensed, peripheral chromatin. Epon; Richardson's stain. (800 ×)

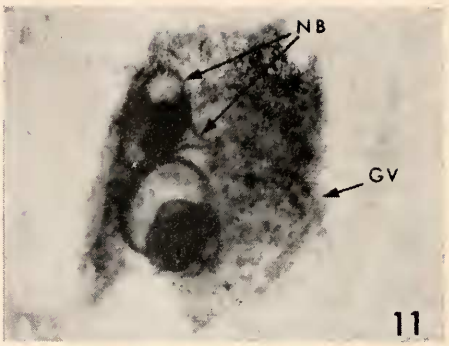
FIGURE 6. *Sp. mörchi:* cross-section through the gonad of a female segment showing an oocyte in pachytene (Pa). Note the discrete nature of the gonad and the thin ventral epidermis (E). Epon; Richardson's stain. (800 ×)

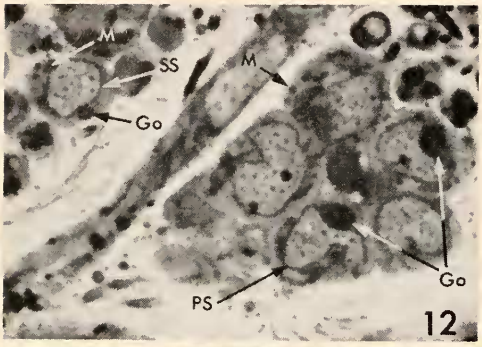
FIGURE 7. *Sp. mörchi:* oblique cross-section through the achaetous region and first abdominal segment showing a progressive series of oocytes extending dorsally from the gonad (G) within the septum (S). Also illustrated are the ventral nerve cord (VN) and ventral ciliated peritoneum (CP). Paraffin; haematoxylin-eosin. (320 ×)

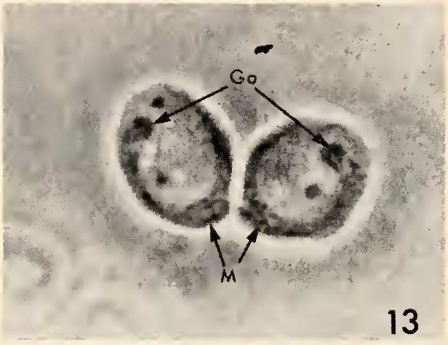
FIGURE 8. *Sp. mörchi:* oblique cross-section through two adjacent female segments showing a series of oocytes which have erupted through the septum into the coelom. Note the large amount of coarse membrane present in the cytoplasm, the nucleolar fragments (NF), and possible Golgi material (Go). Only a few proteid yolk granules (YG) are present. Epon; Richardson's stain. (800 ×)

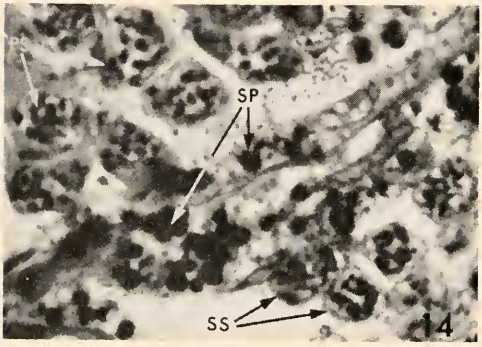
FIGURE 9. *Sp. mörchi:* cross-section through an oocyte showing the eccentric position of the germinal vesicle (GV) and concentric lamellae (CL) of membrane. Note absence of follicle cells. Epon; Richardson's stain. (800 ×)

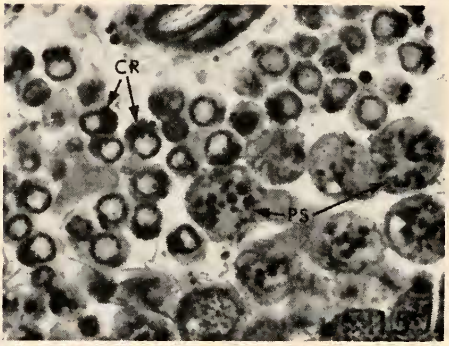


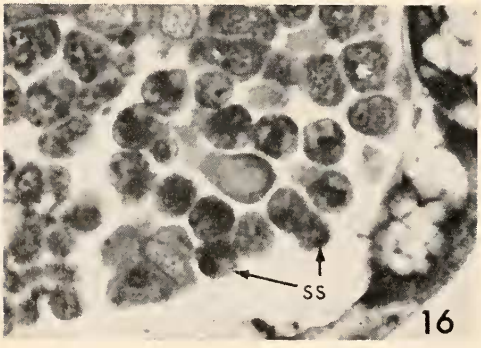


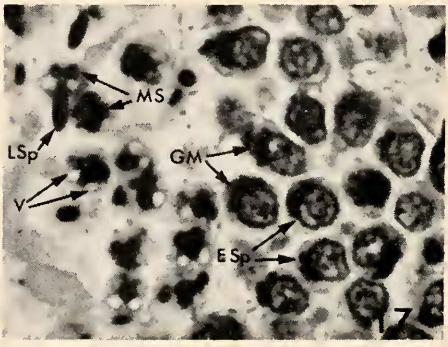












FIGURES 10–17.

The nucleolus in early oocytes is homogeneous, but just prior to vitellogenesis it takes on the staining characteristics of an amphinucleolus. The cortex is acidophilic and the medulla basophilic. As yolk granules begin to appear, the cortex separates into two spherical bodies which retain their identity throughout most of the vitellogenetic period. The medulla also divides and starts to vacuolate and extrude nucleolar material into the nucleoplasm (Fig. 11). Nucleolar vacuolation also occurs in *Sp. spirillum*. The nucleolar products in both species can be observed in germinal vesicles about to be spawned. At the level of light microscopy, there is no evidence for nucleolar extrusion into the cytoplasm.

Bergan (1953a), although he does not describe them, claims to have found abdominal nephridia in the female segments of the four species he examined. He is of the opinion that the dimensions of the nephridia are such that they could serve as genital ducts. It has not been possible to confirm Bergan's observations on local species used in the present study. In *Sp. mörchi*, for example, the ventral peritoneum of the female segments is strongly ciliated but there is no duct arrangement (Fig. 7). This ciliated patch of peritoneum probably represents a remnant of the coelomostome, the habit of shedding oocytes through a coelomoduct having been abandoned. Such remnants as a ciliated flap or patch on the peritoneum are common in polychaetes which release gametes by rupture of the body wall or gut (Dales, 1963). In *Sp. mörchi*, gravid female segments become greatly distended, the ventral body wall measuring about 8 microns in thickness.

Male Segments

Spermatocyte development

The mitotic events associated with primordial germ cell proliferation in the male gonad are the same as in the female gonad. As in the female gonad, pre-

FIGURE 10. *Sp. mörchi:* parasagittal section through a female segment showing the large accumulation of lipid droplets within the peritoneal cells (P) and the thin epidermis (E) of the body wall. Epon; Richardson's stain. (630×)

FIGURE 11. *Sp. vitreus:* section through a primary oocyte showing the germinal vesicle (GV) and nucleolar fragments undergoing nucleolar vacuolation (NB). Paraffin; haematoxylin-eosin. (1600×)

FIGURE 12. *Sp. mörchi:* sagittal section through two adjacent male segments showing a cluster of primary spermatocytes (PS) and a secondary spermatocyte (SS). Note the spherical Golgi material (Go) and mitochondria (M). Epon; Richardson's stain. (1600×)

FIGURE 13. *Sp. vitreus:* primary spermatocytes showing spherical Golgi material (Go) and cluster of mitochondria (M) at the opposite pole. Living material; phase-contrast. (1600×)

FIGURE 14. *Sp. mörchi:* sagittal section through two adjacent male segments showing primary spermatocytes (PS) just prior to first meiotic metaphase and secondary spermatocytes (SS) just prior to second meiotic metaphase. Note inclusions in septal peritoneum (SP). Epon; Richardson's stain. (1600×)

FIGURE 15. *Sp. mörchi:* section through a male segment showing primary spermatocytes (PS) in first meiotic metaphase and spermatids in the "complete ring stage" (CR). Epon; Richardson's stain. (1260×)

FIGURE 16. *Sp. mörchi:* section through a male segment showing secondary spermatocytes (SS) in second meiotic metaphase. Epon; Richardson's stain. (1260×)

FIGURE 17. *Sp. mörchi:* section through a male segment showing early spermatids (ESp) and late spermatids (LSp). Note the granular mitochondria (GM) concentrated at one pole in the early spermatids and fusion of mitochondria to form four mitochondrial spheres (MS) in the late spermatids. The four vacuoles (V) found in the sloughing cytoplasm of the late spermatids alternate with the four mitochondrial spheres. Epon; Richardson's stain. (1925×)

meiotic stages of prophase are difficult to find, only leptotene having been observed on the male side. Also, as in the female gonad, there are a number of cells which have the appearance of cells undergoing degeneration; however, as in the case of the female side, there is no clear evidence that these cells actually degenerate. The primary spermatocyte erupts through the peritoneum of the septum and enters the coelom with the nucleus in the "diffuse state." The fact that growth of the primary oocyte is initiated in the gonad probably accounts for the disparity in size of gonad between the two sexes.

In the coelom, the primary spermatocyte reaches a diameter of about 8 microns. The nucleus contains a fine chromatin network and a single nucleolus. Thread-like mitochondria are scattered throughout the cytoplasm but are especially concentrated at one pole of the cell. At the opposite pole there is a spherical structure which, in osmium-fixed material, is partially or completely surrounded by a sheath of dark-staining material (Fig. 12). The same structure has also been observed in living primary spermatocytes by means of phase-contrast (Fig. 13). It is assumed that the spherical body is made up of Golgi material. Just prior to metaphase I, the chromosomes appear as distinct bivalents and the spherical body breaks up (Fig. 14). The diplotene chromosomes contract greatly as they enter diakinesis but unequivocal chiasma formations have not been observed. With the conclusion of diakinesis, the chromosomes become arranged in metaphase (Fig. 15) and the first meiotic division proceeds. Counts made from sectioned material and aceto-orcein squashes indicate the haploid number to be about 10. Dasgupta and Austin (1960) in five species of *Spirorbis* (*Sp. borealis, Sp. corallinae, Sp. pagenstecheri, Sp. spirillum,* and *Sp. tridentatus*) have found a uniform count of $2n = 20$.

Secondary spermatocytes, resulting from the first meiotic division, enter into an interphase condition. The single nucleolus and delicate chromatin network reappear in the nucleus and the entire diameter of the cell averages 5.7 microns. Thread-like mitochondria remain more or less concentrated at one pole while at the opposite pole dense-staining bodies reaggregate to form the spherical body which is assumed to be of Golgi material (Fig. 12). With the onset of prophase, the nucleolus disappears and prophase chromosomes appear (Fig. 14). After contracting to some extent, the chromosomes become arranged along the metaphase plane and the second meiotic division occurs (Fig. 16).

Spermiogenesis

The early spermatid resulting from telophase II has a total diameter of 3.3 microns. A delicate chromatin net fills the nucleus and granular mitochondria are concentrated at one pole within the scanty cytoplasm (Fig. 17). With the initiation of spermiogenesis, the chromatin goes through a series of complex changes. At the beginning of the cycle, the chromatin pulls away from the center of the nucleus and starts to condense around the periphery just under the nuclear membrane. Further peripheral condensation gives rise to what may be referred to as the "interrupted ring stage." The mitochondria in the latter stage are still granular in appearance and concentrated at one pole (Fig. 18). The next stage in the developmental sequence is characterized by completion of the chromatin ring and is referred to here as the "complete ring stage." At this stage, the mitochon-

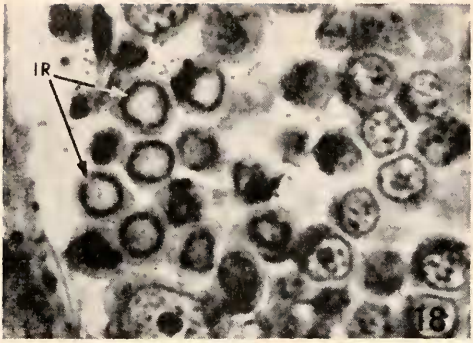
dria have fused into four spheres of uniform size which surround the point from which the tail filament emerges (Fig. 15).

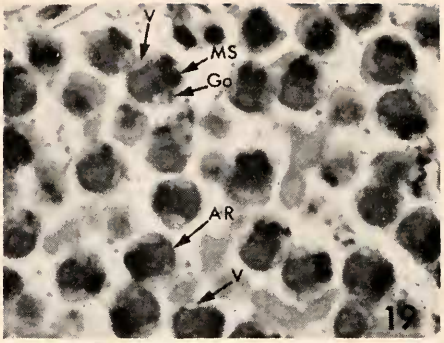
In material fixed in buffered osmium and stained with Richardson's stain, the chromatin ring stains very intensely while the center of the nucleus stains hardly at all. With further development, the chromatin ring disappears except for a dark-staining apical rim, and the nucleus becomes homogeneous. It has not been possible to resolve the events involved in acrosome formation; however, in the last mentioned stage, a dark-staining body is found in the cytoplasm at one side of the developing sperm head and may represent Golgi material (Fig. 19). The dark-staining apical rim might be interpreted to be the deposited acrosome. After becoming homogeneous, the nucleus takes on a conical shape and starts to elongate (Fig. 20). As the head continues to elongate, the cytoplasm moves backward so as to encompass more and more of the tail filament. Four clear vacuoles appear in the apical portion of the sloughing cytoplasm just above the mitochondrial spheres. A cross-section taken through this region of the developing sperm reveals that the vacuoles alternate with the four mitochondrial spheres (Fig. 17). The vacuoles occur in all four species examined and are undoubtedly the "neutral red vacuoles" described by Franzén (1956). The significance of the vacuoles is not known.

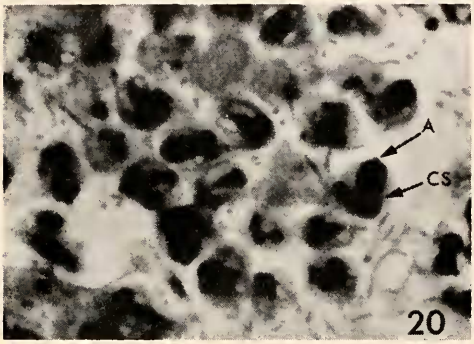
Late spermatids in various stages of cytoplasm elimination form large plates when artificially released into the sea water. This seems to be due to a mutual stickiness and not to the presence of a cytophore or nurse cell arrangement; at least if such a system is present, it is not resolved at the light level. In the last phase of cytoplasm elimination, the four mitochondrial spheres move posteriorly along the tail filament to form the middle piece. In mature sperm, the middle piece becomes homogeneous. The vacuoles are lost with the residual cytoplasm.

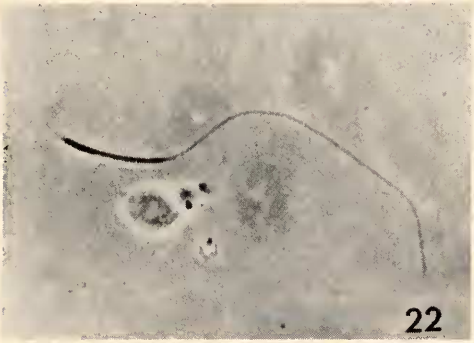
The morphologically mature sperm of *Sp. mörchi* has a cylindrical head capped with a distinctly pointed acrosome and has a total length of about 45 microns. Sharply delimited from the head is the middle piece which is about three times the length of the head. The tail filament is of the ordinary type and is just a little more than twice the length of the middle piece (Fig. 21). In *Sp. ambilateralis* the head of the sperm is also cylindrical but the acrosome is neither as distinct nor as pointed as in *Sp. mörchi*. The middle piece is 1½ times the length of the head and the tail filament has a length equal to the total length of a *Sp. mörchi* sperm (Fig. 22). The average total length of a *Sp. ambilateralis* sperm is 57 microns.

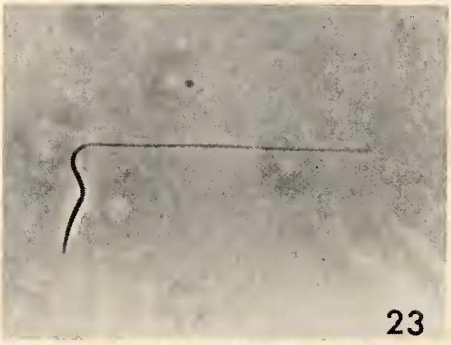
In the two dextral species, *Sp. vitreus* and *Sp. spirillum*, the sperm are morphologically quite different from those of the two sinistral species just described. In both the dextral form and polymorphic sinistral form of *Sp. vitreus*, the sperm head has the shape of a long, slightly bent, pointed cone, a middle piece slightly less in length but of about the same thickness as the head, and a tail filament with a length a little more than twice the combined length of head and middle piece (Fig. 23). The sperm head of *Sp. spirillum* is longer, more sharply bent, and more pointed than that of *Sp. vitreus*. The middle piece is similar but the tail filament is considerably longer, having a length equal to that of an entire *Sp. vitreus* sperm (Fig. 24). In neither species is the acrosome clearly delimited from the head. The average total length of a *Sp. vitreus* sperm is 42.5 microns, whereas

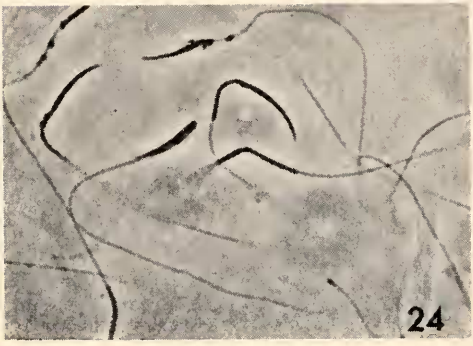


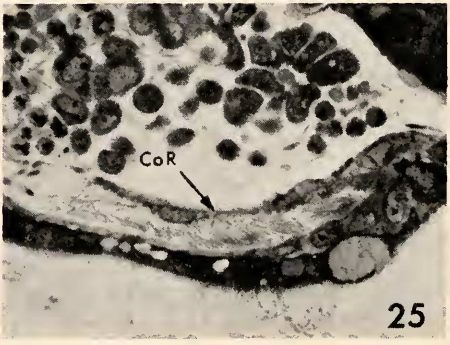












FIGURES 18–25.

the average length of a *Sp. spirillum* sperm is 59 microns. The descriptions for the dextral species are essentially in agreement with those given by Franzén (1956).

Arrangement of stages within the male segments

Each male segment in a sexually mature animal contains stages of spermiogenesis. Synchronous stages are found in clusters within the coelom, but there is no predictable arrangement and each segment is autonomous. Generally, a single segment contains clusters of primary spermatocytes, secondary spermatocytes, and spermatids; however, a single segment may or may not also contain meiotic figures and mature sperm. As one would expect, the synchronous clusters of primary spermatocytes are smallest in size, the cluster size reaching a maximum with the spermatids and sperm. To account for this arrangement, there must be a simultaneous proliferation of primary spermatocytes occurring periodically in each male segment throughout the sexual period.

There is no clear evidence, at the level of light microscopy, for the existence of nurse cells in male segments. It is interesting to note, however, that the peritoneal cells lining the coelomic cavity accumulate inclusions not unlike those found in the peritoneal cells of the female segments (Fig. 14). This accumulation of inclusion bodies never reaches the degree observed in female segments.

The septa separating the male segments are, like those of the female segments, made up of two thin epithelial layers of peritoneal origin. In each half of the male segments, the peritoneal layer of the posterior septum is folded in upon itself in such a manner as to form a short duct near the ventral floor of the coelom. Each duct, the ventral portion of which is ciliated, ends blindly at the same level and just lateral to the ventral nerve cords (Fig. 25). Mature spermatozoa are often found in the ducts, but stages of spermatogenesis from primary spermatocytes to late spermatids may also be found in the ducts. Bergan (1953a) refers to these as abdominal nephridia but this can hardly be correct since their peritoneal origin is obvious. It seems most likely that the ciliated ducts represent remnant coelomostomes.

Discussion

The most striking feature concerning the secondary segments of *Spirorbis* is the fact that each functions as a genital segment and contains a well defined and

Figure 18. *Sp. mörchi:* section through a male segment showing spermatids in the "incomplete ring stage" (IR). Epon; Richardson's stain. (1925 ×)

Figure 19. *Sp. mörchi:* section through a male segment showing spermatids in the "homogeneous stage." Note the apical rim (AR), mitochondrial spheres (MS), forming vacuoles (V), and dark-staining body which may be Golgi rest (Go). Epon; Richardson's stain. (1925 ×)

Figure 20. *Sp. mörchi:* section through a male segment showing spermatids in the "conical stage" (CS). Note that the acrosome (A) is now visible. Epon; Richardson's stain. (1925 ×)

Figure 21. *Sp. mörchi:* nearly mature sperm. Middle piece still somewhat irregular in appearance. Living material; phase-contrast. (1140 ×)

Figure 22. *Sp. ambilateralis:* mature sperm. Living material; phase-contrast. (1140 ×)

Figure 23. *Sp. vitreus:* mature sperm. Living material; phase-contrast. (1140 ×)

Figure 24. *Sp. spirillum:* mature sperm. Living material; phase-contrast. (1140 ×)

Figure 25. *Sp. mörchi:* cross-section through a male segment showing a coelomostome rudiment (CoR). Epon; Richardson's stain. (700 ×)

persistent gonad. Distinct gonads have been described in *Salmacina dysteri* (Malaquin, 1925), *Filograna implexa* (Faulkner, 1930), *Pomatoceros triqueter* (Thomas, 1940; Jyssum, 1957), and in the Arenicolidae (Fauvel, 1959; Matthews, 1962). With the exception of the forms just mentioned, a discrete gonad is not characteristic of polychaetes. In a majority of polychaetes the germ cells arise from rather indefinite patches of peritoneum (Parker and Haswell, 1957; Fauvel, 1959; Dales, 1963).

Cells having features similar to those of the primordial germ cells in *Spirorbis* have been reported in the three serpulid species mentioned above (Malaquin, 1925; Faulkner, 1930; Jyssum, 1957). Jyssum (1957) in a study on oogenesis in *Pomatoceros triqueter* refers to these cells as neoblasts and describes them as giving rise to the female gamete. The interphase nuclei of the neoblasts contain peripheral chromatin clumps; however, they are not as regular in size and form as are those of the primordial germ cells in *Spirorbis*. Another difference between the two is that the neoblasts contain one or two nucleoli. Jyssum describes the neoblasts as dividing and giving rise to gonia which, in turn, divide to give rise to primary oocytes. The distinction between the two divisions is apparently based on the thickness of the chromosomes at metaphase, the chromosomes of the neoblasts being thicker and more lumpy than the chromosomes in gonial metaphase. Such a gonial stage, between primordial germ cell and primary cyte, has not been observed in *Spirorbis*. A distinct gonial stage in *Spirorbis* is quite probable, but, as yet, has not been identified. It will be shown, in a subsequent report, that the primordial germ cells arise relatively early in the development of *Spirorbis* and can be followed to the sites of gonad formation in the metamorphosed pre-adult.

Many of the problems associated with oogenesis have already been discussed. The fact that most of the growth and maturation of the oocyte occur freely within the coelom without the aid of attached follicle or nurse cells offers a number of possibilities for experimental investigation of this type of oocyte development. For example, it may be possible to culture oocytes *in vitro* and study the effects of environmental conditions on growth and vitellogenesis.

There are a number of problems associated with spermiogenesis in *Spirorbis* which are beyond the resolution afforded by the light microscope. It would be of interest, for example, to study acrosome formation. Only a few electron microscope studies of acrosome formation in invertebrates have been attempted (see Cameron and Fogal, 1963) and some of the homologies between various types of acrosomes are at a start of being elucidated. Another problem which would be of interest is the origin and possible significance of the "neutral red vacuoles" which are so apparent during spermiogenesis.

The mature sperm of *Spirorbis* is not of the simple or primitive type but is modified. Two morphological types have been described here. Franzén (1956) recognizes three morphological types in the genus: *Sp. spirillum* and *Sp. vitreus* share one type; *Sp. borealis* and *Sp. granulatus* share another type quite different from the first; and a third type, even more highly modified than the other two, is found in *Sp. pagenstecheri*. The descriptions given in the present study for *Sp. spirillum* and *Sp. vitreus* agree in the main with those given for the same species by Franzén. Sperm morphology of *Sp. mörchi* and *Sp. ambilateralis* is not consistent with any of the three types recognized by Franzén. There can be

no doubt that the sperm morphology in the genus *Spirorbis* has some usefulness as an auxiliary systematic character. In this connection it may again be pointed out that the sperm morphology of the sinistral form of *Sp. vitreus* is identical with that of the dextral form.

Franzén's major thesis is that there is a definite relation between the morphology of the sperm and the biology of fertilization. According to this thesis, invertebrates which discharge their gametes freely into the water retain a primitive type of sperm which is characterized as consisting of a short rounded to conical head, a small middle piece containing four to five mitochondrial spheres, and a tail formed by a long flagellum. Invertebrates which have an altered biology of propagation exhibit a modified sperm morphology. If the end product of spermatogenesis is a modified sperm, primitive characters are retained during spermiogenesis; the four mitochondrial spheres which appear in the spermatid of *Spirorbis* would be such a character. In a discussion of the family Serpulidae, Franzén points out the hermaphroditic and brooding nature of *Spirorbis* but admits that literature on the reproductive biology of the genus is extremely incomplete. Speculating on the mode of fertilization, he is of the opinion that the most natural way for it to occur would be that sperm from a nearby animal are sucked into the tube and there fertilize the eggs. If this were the case, sperm would not have to swim great distances in order to reach the eggs. There is also evidence that at least certain species of *Spirorbis* are capable of self-fertilization (Potswald, 1964; Gee and Williams, 1965).

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Summary

1. In all species of *Spirorbis* examined, the first two or three abdominal segments of mature adults are female and the remaining abdominal segments are male. Both female and male gametes differentiate simultaneously in the same individual and arise from a discrete and persistent gonad composed of primordial germ cells arranged in two retroperitoneal rows, mesial to the ventral nerve cords, and running the length of the abdominal segments.

2. Cytological events associated with the development of female and male gametes are described. Differentiation of oocytes and spermatocytes occurs freely within the coelomic cavity without the aid of attached nurse cells.

3. Although coelomostome rudiments are present in both female and male segments, functional genital ducts do not develop. Spawning is assumed to take place by rupture of the body wall.

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